*Acta Oncologica* Vol. 34, No. 3, pp. 397–400, 1995

# THE MODIFICATION OF BLOOD FLOW IN TUMOURS AND THEIR SUPPLYING ARTERIES BY NICOTINAMIDE

DAVID G. HIRST, GORDON D. KENNOVIN, GILLIAN M. TOZER, VIVIEN E. PRISE and ERIC W. FLITNEY

**We have studied the ability of the radiosensitizer nicotinamide (NA) to alter the contractility of normal and tumour blood vessels using an ex vivo isolated artery perfusion system. NA at a concentration of 8.2 mM reduced the constrictions produced by phenylephrine (PE) by 2-fold in both normal epigastric arteries and those that had been supplying p22 tumours in BD9 rats. At that same concentration NA also attenuated the spontaneous, rhythmic contractions that were seen in many tumour arteries. When the tumour arteries were perfused together with the tumour they supplied NA had little effect on the flow resistance of the tumour vascular network but reduced the resistance by up to 30% when the arteries were preconstricted with phenylephrine. These direct effects on vascular resistance together with the reduction of interstitial fluid pressure could combine to improve the homogeneity of tumour perfusion.**

Following extensive preclinical studies (1) of the ARCON (accelerated radiotherapy with carbogen and nicotinamide) concept, nicotinamide (NA), the amide derivative of vitamin $B_3$, is currently being tested in four separate EORTC clinical trials, in combination with radiotherapy for cancer of the lung, bladder, brain, and head and neck. NA was always seen as a lead compound and a very comprehensive programme was pursued to develop better, structurally-related, analogues (2) using tumour radiosensitization as the main end point. While it was possible to relate structure to activity in this study, the search for a better compound was hindered by the lack of understanding of the basic mechanism of action of NA and none of the analogues tested proved to be significantly more effective at sensitizing tumours to radiation.

The first indication that nicotinamide (NA) might be useful in the treatment of cancer came from reports that, at high doses, it could specifically sensitize transplanted murine tumours to radiation (3, 4). It was initially thought that radiosensitization by NA was a consequence of the drug's ability to inhibit the repair of radiation-induced DNA damage, as had been demonstrated in vitro (5), but it soon became apparent that a major part of the sensitizing effect resulted from changes in tumour oxygenation (6). Further investigation has since shown that NA acts mainly to reduce the microregional heterogeneity of tumour blood flow (7). This highly specific mode of action has prompted us and others to investigate the underlying mechanisms which remain poorly understood. We have used isolated arteries from normal and tumour tissues in the rat to study the direct action of NA.

## Material and Methods

*Drugs.* NA and phenylephrine (PE) were obtained from Sigma Chemical Company (Poole, Dorset) and were freshly made up each day in Krebs' solution. The composition of the Krebs' solution and the dilution of the drugs has been described previously (8).

Received 23 September 1994.
Accepted 7 December 1994.
From the Department of Biomedical Sciences, University of Ulster, Newtownabbey, Co. Antrim, Northern Ireland (D.G. Hirst), Cancer Biology Research Group, Cell Biology and Neuroscience Research Division, University of St. Andrews, St. Andrews, Fife (G.D. Kennovin, E.R. Flitney) and CRC Gray Laboratory, Mount Vernon Hospital, Northwood, Middx. (G.M. Tozer, V.E. Prise), U.K.
Correspondence to: Professor David G. Hirst, Department of Biomedical Sciences, University of Ulster, Newtownabbey, Co. Antrim, Northern Ireland BT37 0QB, U.K.
Paper presented at the Tumour Microenvironment Workshop in Granada, Spain, September 23–24, 1994.

 ISSN 0284-186X

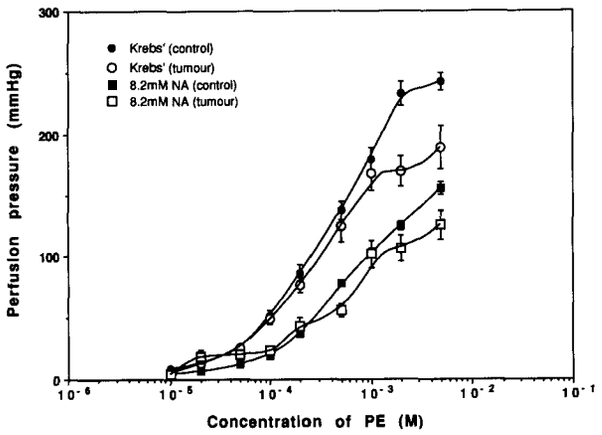


*Fig. 1.* Dose response curves for the constriction of epigastric arteries by phenylephrine in an ex vivo perfusion system. Normal arteries and those that had previously supported tumour growth in vivo were perfused in the presence or absence of 8.2 mM nicotinamide. Error bars show ±1 SEM; n = 9.

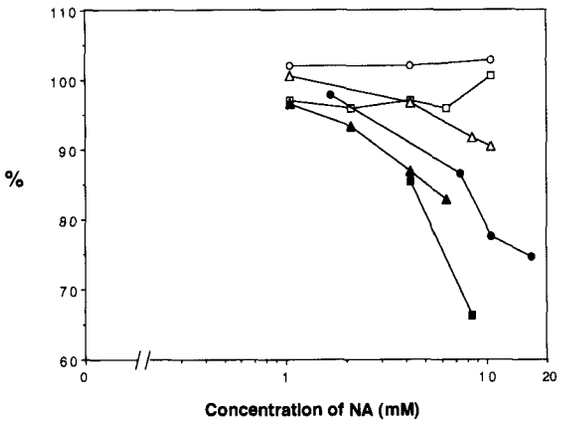


*Fig. 2.* Flow resistance in three whole P22 tumours, perfused through their supplying epigastric artery, as a function of nicotinamide concentration. Tumour vessels were perfused either in a relaxed condition (open symbols) or were preconstricted by adding $4 \times 10^{-7}$ M phenylephrine to the perfusate (closed symbols). Different symbols were used for individual tumours.

*Animals and tumour system.* The P22 adenocarcinoma growing in the male BD9 rat was used in all experiments. We have described the origin and passaging of the tumour in a previous publication (9). Small pieces of tumour obtained from a donor animal were implanted in the right inguinal fat pad and allowed to grow to a size of 0.5–1g at which time the tumour, complete with its blood vessels, was excised after cannulating the afferent artery. These procedures have previously been described in detail (9). A simplified protocol was used in some of these experiments investigating only the arteries, without attached tumours. The details have been given in a previous publication (10).

*Ex vivo perfusion.* The cannulated tissues to be investigated were placed in an apparatus that allowed perfusion of the arteries and in one series of experiments the whole tumour, with a variety of solutions. A detailed description of the apparatus used in these experiments has been given in our previous publications (8–10). Briefly, the perfusate was forced through the preparations using a constant flow pump and the back pressure measured with a pressure transducer and recorded on a computer; increased pressure therefore represented an increase in flow resistance (vasoconstriction) while decreased pressure was the result of vasodilatation.

*Experimental design.* The aim of the present study was to determine if NA had such potent vasorelaxant effects on the arteries that supply tumours as that which we had observed in the normal rat arteries (8). In that study, two different protocols had been used. In one, transient vasoconstrictor responses were elicited by bollus injections of phenylephrine (10 $\mu$l of $10^{-5}$–$10^{-2}$ M) and dose response curves generated. These curves were then modified by the addition of NA to the perfusate. In protocal 2, the test arteries were preconstricted with a constant perfusion of 5 $\mu$M PE and then attenuated by the addition of a range of NA concentrations to the perfusate. In the experiments described in the present paper protocol 2 could not be used effectively on isolated tumour arteries because of their tendency to undergo spontaneous rhythmic constrictions. This spontaneous activity was also seen in whole P22 tumours perfused ex vivo but, for reasons we do not yet understand, it occurred less frequently and it was possible to conduct a series of experiments using this preparation.

## Results

*Inhibition of transient vasoconstrictor responses to PE by NA.* PE dose response curves were constructed for both tumour-supplying and normal epigastric arteries (nine of each) in the presence or absence of 8.2 mM NA. As shown in Fig. 1 both tumour and normal arteries constricted progressively with increasing PE concentration though the peak constriction was restricted in the tumour arteries. When NA at a concentration of 8.2 mM was added to the perfusate, constriction was attenuated. The magnitude of the effect was dependent on the PE concentration and was similar in tumour and normal arteries. For example, a concentration of $2 \times 10^{-3}$ M PE gave a perfusion pressure in normal arteries of 235 mmHg, but when NA was added to the perfusate the same PE concentration gave a pressure of only 125 mmHg, a reduction by a factor of 1.9. In terms of PE dose, the normal arteries required only $4 \times 10^{-4}$ M in the absence of NA to produce a pressure of 125 mmHg, a dose reduction factor of 5.

*Inhibition of tumour-pecific spontaneous rhythmic contractions.* We have previously reported 'spontaneous' rhythmic contractions in tumour vessels (10). This activity created difficulties in carrying out experiments of the kind shown in Fig. 2 where a stable baseline is required. However, this rhythmic activity was an interesting phenomenon

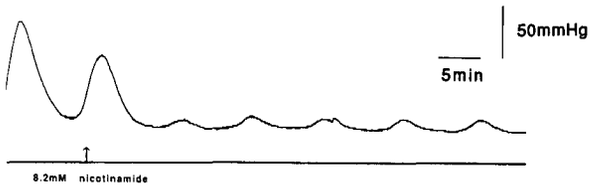


*Fig. 3.* An example of the spontaneous fluctuations in pressure seen in a tumour supply artery perfused at a flow rate of 0.6 ml/min and the inhibition of that activity by 8.2 mM nicotinamide.

in its own right and so we investigated the effect of NA (8.2 mM) on spontaneously contracting, isolated epigastric arteries that had been supplying P22 tumours. NA inhibited, but did not abolish, spontaneous rhythmic contractions in tumour-supply vessels in all 8 arteries tested. An example is shown in Fig. 3. The inhibition was rapid, occuring within 1 min of the drug contacting the artery.

*Vascular relaxation in preconstricted tumours by NA.* Mean vascular resistance (in $mmHg.ml^{-1}.g^{-1}.min^{-1}$) in non-preconstricted tumours was 209; in tumours preconstricted with PE mean resistance was 409. Fig. 2 shows the ability of a range of NA doses to cause a reduction in flow resistance (normalized to the value prior to NA exposure) in three P22 carcinosarcomas (weight range 466–866 mg) perfused through their supplying artery. NA had very little effect when the tumour vessels were relaxed, but resistance was reduced by 15–35% at a NA concentration of 8 mM when they were preconstricted with PE.

## Discussion

There is now considerable evidence that NA affects blood flow both in whole tumours and at the microregional level (12–14). It is also clear that NA, when given at a high enough dose will markedly reduce blood pressure in rodents (15–18). Blood flow in rodent tumours has been measured by a wide variety of different methods. The results obtained in nearly all studies show a small but consistent increase in tumour blood flow with NA doses in the range 400–1 000 mg/kg. We know that NA doses in this range substantially reduce blood pressure in mice so the only way to explain an increased flow is to postulate that NA produces a larger reduction in flow resistence in tumour vessels than it does in the normal vasculature. The experiments described in this paper were an attempt to establish if this is true. As can be seen in Fig. 1, the tumour vessels were less sensitive to the vasoconstrictive effects of high concentrations of PE, a phenomenon we have reported previously ((10) but NA was equally effective at inhibiting the PE-induced vasoconstriction at the concentration used (8.2 mM)). Thus, we were unable to demonstrate any tumour specificity for the action of NA, at least in these main supply arteries. We already know that the main supplying artery contributes little to the overall flow resistance of the tumour vasculature in this system (9) so that the effect of NA shown in Fig. 2 is probably dominated by its action on vessels within the tumour. A concentration of 4 mM NA reduced flow resistance by about 12% which would result in a 12% increase in tumour blood flow if there was no change in perfusion pressure. That increase is rather less than has been measured in rodent tumours in vivo using a variety of methods (13, 14). Furthermore, we know that 4.1–5 mM NA in vivo reduces systemic blood pressure by between 15 and 25% in rats (15) and mice (16–18) which should negate the reduction in flow resistance we have observed.

The results reported here do not support the hypothesis that tumour vasculature is more sensitive than normal vasculature to the vasorelaxant action of NA. It is possible that the isolated perfused tumour as used in our ex vivo system does not accurately reflect the sensitivity of the tumour vasculature in vivo. We believe, however, that the mechanism of action of NA in tumours could be explained, not so much in terms of the steady state pressure/flow relationships of the tissue but in terms of reduced heterogeneity of flow distribution. In our system, the vessels that supplied the tumours exhibited a tendency to undergo spontaneous rhythmic contractile activity, a phenomenon which could not be elicited in the normal counterpart (10). Taken together with another characteristic of tumours, elevated interstitial fluid pressure (IFP), this activity might offer an explanation for heterogeneity of tumour blood flow (19). In normal tissues intravascular pressure (IVP) is always significantly higher than the pressure in the interstitium so there is little chance of vessels being compressed by the surrounding parenchyma, even if perfusion pressure fluctuates as it would in response to vasomotion. In tumours, however, IFP is high and relatively uniform throughout the tumour mass (20); therefore, IFP may be very close to IVP at the venous end of the tumour vascular network so that even a small reduction in perfusion pressure resulting from transient constriction at the arterial end could cause temporary occlusion at the venous end. Thus, an elevated IFP should amplify the consequences of vasomotion. This process may be further enhanced by large, spontaneous, rhythmic constrictions as we have observed in our ex vivo preparations of tumour blood vessels (10). By reducing IFP, therefore, NA could reduce the severity of the consequences of vasomotion on tumour perfusion; furthermore, as shown in Fig. 3, NA can also attenuate oscillatory vasoconstrictions. This combination of actions could offer an explanation for the remarkable ability of NA to reduce hypoxia that results from microregional stoppages in blood flow without greatly increasing overall tumour perfusion.

The peak plasma levels of NA that can be achieved currently in man are around 1 mM. While physiological effects in rodents are minimal at this dose there is undoubtedly significant radiosensitization of mouse tumours

(21). We may ask, therefore, if the effects we have reported here have any relevance to potential radiosensitization of human tumours. At present we have insufficient data to determine whether a dose of $\sim 1$ mM produces a small reduction in vascular resistance or no effect at all. If it is a small but significant effect the influence of elevated IFP may be sufficient to amplify its importance in certain tumour regions, giving rise to a significant reduction in the number of perfusion-limited hypoxic cells. Furthermore, a full dose response curve has not yet been generated for the inhibitory action of NA on spontaneous contractile activity (Fig. 3) and it is possible that this may be an important contribution to the radiosensitizing effect.

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